Effects of Lead and Copper Exposure on Growth of an Invasive Weed, *Lythrum salicaria* L. (Purple Loosestrife)[1]

ANN M. NICHOLLS AND TARUN K. MAL[2], Department of Biological, Geological, and Environmental Sciences, Cleveland State University, 2399 Euclid Avenue, Cleveland, OH 44115

ABSTRACT. The concentrations of heavy metals such as copper and lead in the environment are currently increasing, due mainly to human activities. Any of these metals, at sufficiently high concentrations, can cause severe damage to physiological and biochemical activities of plants. This damage to plants can be intensified when two or more metals present in the soil act synergistically. This study assessed the effects of two metals, copper and lead, on the growth of an invasive plant species, *Lythrum salicaria*. Treatments consisted of control; high and low concentrations of lead; high and low concentrations of copper; and high and low concentrations of both copper and lead. Although the treatment of plants with metals significantly reduced growth and survival of the plants, no synergistic relationship between the metals was found because of the extreme toxicity of the concentrations of metals used in this investigation.

OHIO J SCI 103 (5):129–133, 2003

INTRODUCTION

Anthropogenic pollutants such as heavy metals enter our environment in a variety of ways. These include mining, metal smelting, electroplating, gas exhaust, energy and fuel production, down wash from power lines, intensive agriculture, power transmission, sludge dumping, and military operations (Kumar and others 1995; Nedelkoska and Doran 2000). Because of these activities, the levels of heavy metals, such as lead and copper, in the environment are currently of great concern (Cooper and others 1999). Although some of these metals, such as copper and zinc, have known functions as micronutrients and are needed by plants as parts of coenzymes and enzymatic prosthetic groups (Antosiewicz 1992), lead and mercury have no known biological functions (Antosiewicz 1992; Xiong 1998). Whether they are required for plant growth or not, all heavy metals are toxic to plants at high levels (Antosiewicz 1992; Nedelkoska and Doran 2000).

The levels of metals found in plants are often correlated to the levels present in the environment (Vesk and Allaway 1997). For example, Salim and others (1993) showed that the concentrations of lead, cadmium, and copper increased in radish plants when treated with an increasing concentration of these metals. The absorption of metals from the soil by plants is influenced by a variety of factors, including pH, temperature, soil ions, the cation exchange capacity of the soil, organic matter content of the soil, the type and concentration of metal, and the species of plant (Antosiewicz 1992; Salim and others 1993). The metals enter the root in the form of dissolved ions and move with the inflow of water apoplastically through the root hairs and into the cortex and are then translocated to other parts of plants (Punz and Sieghardt 1993).

Heavy metals act as stressors to plants when encountered in the environment (Baker 1987). They interact with the physiological and biochemical activities of plants to reduce their vigor and in extreme cases can completely inhibit growth (Baker 1987). Numerous studies have demonstrated the deleterious effects of copper on plant growth (Salim and others 1993; Zhu and others 1999; Mal and others 2002a). Lead also exerts negative effects on both growth and leaf expansion of plants (Kumar and others 1995; Uveges and others 2002). Lead has been shown to have toxic effects on a variety of metabolic processes essential to plant growth and development, including photosynthesis, transpiration, DNA synthesis, and mitotic activity (Wierzbicka 1999). Of these processes, photosynthesis was found to be the most sensitive to lead contamination (Singh and others 1997).

Lead has been acknowledged to be the most abundant metal pollutant in the environment (Watanabe 1997; Xiong 1998). The level of lead in the environment has increased significantly as a result of industrialization and urbanization (Singh and others 1997). Since the half-life of lead in biological systems is one of the longest among metals, the consequence of lead pollution can be far-reaching and devastating (Lane and Martin 1977; Kumar and others 1995). The half-life of lead is estimated to be 150-5000 years (Kumar and others 1995).

Lead occurs in soils in the form of insoluble and soluble salts (Singh and others 1997). It tightly binds to organic soil particles (Kumar and others 1995; Singh and others 1997), which may decrease the mobility of lead in most soils and may reduce uptake by plants (Salim and others 1993; Kumar and others 1995; Cooper and others 1999). It has been suggested that the mobility of lead and copper is greater in sandy soils, which tend to lack organic matter, than in organic soils.

Copper, unlike lead, is necessary for the growth of plants (Cook and others 1997). It functions as a structural and catalytic component of several enzymes and proteins (Cook and others 1997). Despite this requirement, excess copper when absorbed by plants can be toxic, leading to reduced growth, chlorosis, and malformation of roots (Kjaer and Elmegaard 1996; Cook

[1]Manuscript received 2 January 2002 and in revised form 23 September 2002 (#02-04).
[2]Corresponding Author: Address same as above; Email: t.mal@csuohio.edu; Phone: 216-687-2444; Fax: 216-687-6972

and others 1997). The amount of copper that is absorbed by plants and its toxicity depends on several factors. For example, the toxicity of copper on plants is dependent on their nutritional status (Pahlsson 1989). In addition, the toxicity of copper is directly related to the exposure time and the concentration of copper in the soil (Pahlsson 1989; Kjaer and Elmegaard 1996). The toxicity also depends on the genotype of a species. Some genotypes can resist damage due to the toxicity of copper, whereas others are more vulnerable (Pahlsson 1989).

Part of the damage that copper inflicts on plants arises from the interaction of excess copper with other nutrients, such as potassium, zinc, magnesium, and iron (Kjaer and Elmegaard 1996; Cook and others 1997). This may be due to either displacement or altered uptake of the elements by plants (Cook and others 1997). The alteration in uptake may come about through the competitive exclusion of other metals by copper at the uptake sites.

We studied the effects of two heavy metals, lead and copper, on the growth of an invasive plant species *Lythrum salicaria* (Mal and others 1992). The treatments were added to the soil in solution with water (control) only, with one metal, or with a mixture of both metals. The mixture of metals was included in order to determine the occurrence of any synergistic interactions between lead and copper on *L. salicaria*. Each metal or combination of metals was added at high and low concentrations in order to determine whether an increase in concentration would lead to an increased toxicity to the plants.

MATERIALS AND METHODS

Lythrum salicaria L. (Lythraceae, purple loosestrife) is an invasive emergent weed of North American wetlands (Mal and others 1992; Mal and others 1997). Open pollinated seeds were collected from a monoculture of *L. salicaria* in LaSalle, Essex County, Ontario, Canada, in 1996. Seeds were germinated in petri dishes on moistened filter paper in a growth chamber in a 16/8 hr light/dark cycle. Individual seedlings were then transplanted to 10 cm pots filled with sand. The plants were allowed to grow in a 16/8hr light/dark cycle in a growth chamber. Liquid fertilizer (Miracle-Gro®, Stern's Miracle-Gro Products, Inc., Port Washington, NY 11050) was added in the recommended dose to each pot every two weeks, and the size of each plant was measured weekly so that temporal changes in growth could be detected.

Either lead, copper, or their mixture was added to different plants in seven groups of treatments: control – no pollutants; high (2000 ppm) and low (1000 ppm) concentrations of lead; high (2000 ppm) and low (1000 ppm) concentrations of copper; and high (1000 ppm lead + 1000 ppm copper) and low (500 ppm lead + 500 ppm copper) concentrations of lead and copper mixture (Azar and others 1973; Mal and others 2002b; Uveges and others 2002). There were 12 individual genotypes within each treatment group. The plants were assigned treatments randomly.

Before treating the plants, the total length of the main axis and all side branches of each plant was measured. Following the treatment, plants were identified as either being dead or alive. Dead shoots were removed and placed in individually tagged paper bags for the analysis of dry-mass. Weekly measurements of shoot length of the surviving plants were taken.

Fifty-five days after treatment, the plants were harvested and dried at 60° C for one week until they reached constant dry-mass. Root and shoot dry-mass were recorded separately. One-way ANOVAs with Tukey post-hoc tests were conducted on initial length, final length, total dry-mass, shoot and root dry-mass. Repeated measures analysis was used to investigate the patterns of change in growth over time among treatments. Statistical analyses were done using SYSTAT (Wilkinson 1998).

RESULTS

The total length of plants from the different treatment groups did not differ prior to the addition of lead and copper treatments (Table 1). Application of lead and/or copper, however, caused complete withering and death of the above ground parts of all plants. No such damage to the shoots was seen in the control plants. Following the treatment and subsequent death of the aboveground parts of the plants, re-growth occurred in several plants that were treated exclusively with lead (Fig. 1). However, the total length of shoots at harvest was significantly greater in the control than in the metal treatments, even when re-growth occurred (Table 1; Fig. 1).

The dry-mass of dead shoots, collected immediately after treatments were applied, was significantly greater in plants exposed to metals than those in the control treatment (Table 1; Fig. 2). The dry-mass of roots and living shoots at harvest was significantly greater in control plants compared to treatment plants (Table 1; Fig. 2). There was no significant difference in the dry-mass of the roots or shoots in the lead and copper treated plants.

Time had a significant effect on growth both before and after treatments were applied (Tables 2, 3). Although there was no interaction of time and treatment before treatment application, a significant interaction was observed on height after treatment application (Tables 2, 3). The control plants continued to grow, while almost all treatment plants stopped growing, except where new shoots emerged (Fig. 3).

DISCUSSION

Heavy metals are thought to be one of the most dangerous stressors that occur in the environment (Szalontai and others 1999). For this reason, there have been numerous studies on the toxicity of heavy metals, including lead and copper (Pahlsson 1989). Heavy metals impede growth of plants, leading to smaller leaves that tend to be chlorotic (Kovacevic and others 1999; Sayed 1999). These effects can be explained physiologically and biochemically. The treatment with heavy metals can lead to the interruption of activities of several essential enzymes, various aspects of the photosynthetic processes, uptake of essential nutrients, and the ultrastructure and water usage of cells (Sayed 1999). For

TABLE 1

Results of ANOVA testing the effects of lead and copper treatments on growth of Lythrum salicaria.

Dependent Variable	Multiple r^2	df	Mean-square	F ratio	P
Total length prior to treatment	0.09	6	0.26	0.73	0.626
Error		40	0.36		
Total length at harvest	0.84	6	36511.62	34.31	0.000
Error		40	1064.19		
Dry mass of living shoots	0.84	6	10.23	33.96	0.000
Error		40	0.3		
Dry mass of dead shoots	0.28	6	2.14	2.63	0.030
Error		40	0.81		
Dry mass of roots	0.75	6	1.55	20.6	0.000
Error		40	0.07		

example, copper can damage the permeability of plasma membranes, which can lead to the leakage of potassium ions and other substances dissolved in the cell (Ouzounidou and others 1992).

The photosynthetic apparatus may be especially sensitive to damage from heavy metals (Kovacevic and others 1999). Wong and Chang (1991) demonstrated that the reduced photosynthetic efficiency can lead to reduced growth in algae. Heavy metals affect many processes vital to the photosynthetic pathway. For example, excess heavy metals have been shown to negatively affect chlorophyll biosynthesis (Ouzounidou and others 1992), to alter the action of Ribulose 1,5-bisphosphate carboxylase-oxygenase (Cook and others 1997), to interfere with the dynamics of the thylakoid membrane (Szalontai and others 1999), and to inhibit electron transport system in both photosystem I and photosystem II (Ouzounidou and others 1992). Any or all of these effects can explain the significant damage and death of the stems and leaves of the chemically treated plants in the present study.

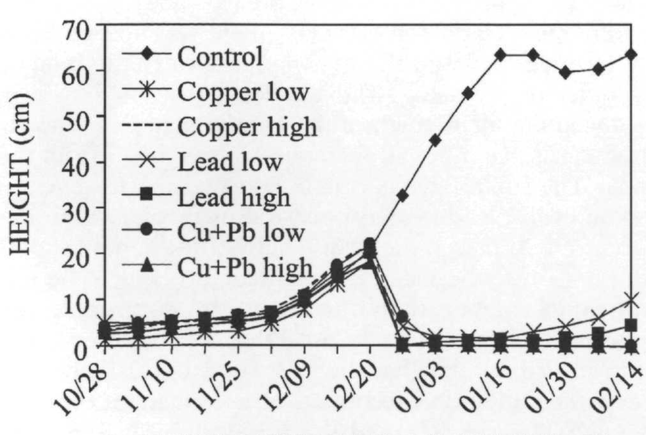

FIGURE 1. Effect of heavy metal exposure on the height of *Lythrum salicaria* during the observation period.

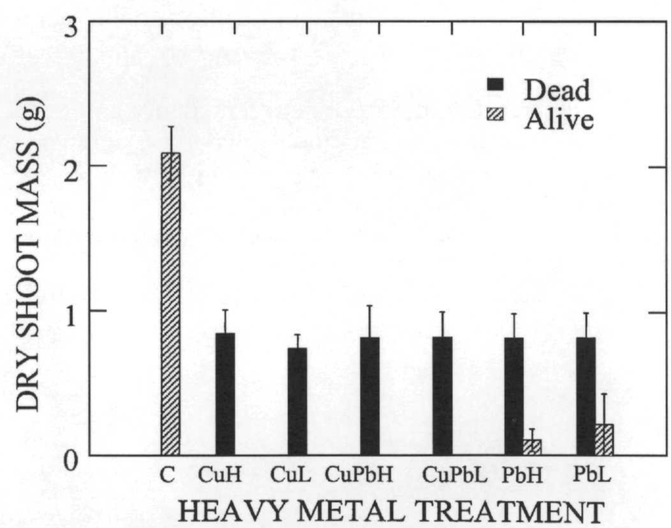

FIGURE 2. Effect of heavy metal exposure on dry biomass of shoots of *Lythrum salicaria* at harvest (C = Control; CuH = Copper high; CuL = Copper low; CuPbH = Copper + Lead high; CuPbL = Copper + Lead low; PbH = Lead high; PbL = Lead low).

In this study, we observed shoot growth and above and below ground biomass of plants in order to test the effects of exposure of these plants to lead and copper. Treatment with lead and copper, irrespective of the concentration of metals, led to complete and rapid death of the above ground stems and leaves. The leaves were affected first, followed shortly by the stems. When and

TABLE 2

Results of repeated measures ANOVA testing the effects of time on height of plants assigned to different treatment groups prior to administering heavy metals.

Source	MS	df	*F*	*P*
Between subjects				
Treatment	16.08	3	0.19	0.9025
Error	84.55	43		
Within subjects				
Time	1666.83	8	324.53	0.000
Time*Treatment	3.05	24	0.59	0.681
Error	5.14	344		

how the roots were affected was not apparent until harvest. In most previous studies, roots were affected most severely when plants were exposed to heavy metals. For example, it has been reported that the growth of roots is the most rapid and sensitive response of plants to pollution with heavy metals (Ouzounidou and others 1992; Romeu-Moreno and Mas 1999). Ouzounidou and others (1992) also observed that the cells of roots are affected more severely by the exposure to copper than are other parts of the plant. Many studies have indicated that the heavy metals are mostly accumulated in the roots rather than in the leaves and stems (Ouzounidou and others 1992; Kovacevic and others 1999).

The difference in the vigor between control and treatment groups was measured and shown to be significant in four ways. Most of the metal-treated plants died. The total length of shoots at the time of harvest was significantly greater in the control plants (also see Uveges and others 2002). The dry-mass of living shoots and roots harvested at the end of the experiment was significantly higher in the control plants.

When a plant is exposed to more than one pollutant, interactions between those pollutants may occur. Although heavy metals may have antagonistic, additive, or synergistic effects in plants, synergistic interactions are found to be most common (Keltjens and van Beusichem 1998; Luo and Rimmer 1995; Wong and Chang 1991; Pahlsson 1989). Synergistic interactions are very important to the ecological processes in the natural world because, in many polluted environments, pollution is caused by more than one pollutant (Keltjens and van Beusichem 1998). This is especially true in areas where sewage sludge is disposed on land or in areas where river and harbor dredgings and metal mining wastes are dumped (Luo and Rimmer 1995). We attempted to simulate a multi-metal contaminated environment in this experiment, but a synergistic relationship was not observed. This may be due to the fact that the metals were added at a level that was too damaging to detect interactive effects of both metals.

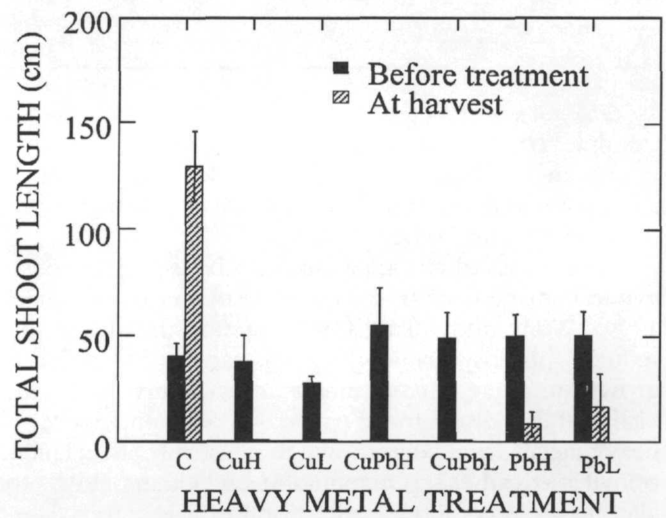

FIGURE 3. Effect of heavy metal exposure on the total shoot length of *Lythrum salicaria* before treatment and at harvest (C = Control; CuH = Copper high; CuL = Copper low; CuPbH = Copper + Lead high; CuPbL = Copper + Lead low; PbH = Lead high; PbL = Lead low).

TABLE 3

Results of repeated measures ANOVA testing the effects of time on height of plants assigned to different treatment groups after administering heavy metals.

Source	MS	df	*F*	*P*
Between subjects				
Treatment	66556.1	3	289.88	0.000
Error	229.56	43		
Within subjects				
Time	2552.45	7	8.92	0.000
Time*Treatment	7758.22	21	9.04	0.000
Error	12298	301		

Prior to the addition of metals, there was no significant difference between the total lengths of plants placed in different treatments. The addition of metals, however, reduced the total length of the treated plants to virtually nothing, since all dead material was removed from the pots. The control plants continued to grow. However, in some of the lead-treated pots, regrowth of new shoots occurred. Although the total length of these new shoots did not cause the mean of lead-treated plants to be significantly higher than the mean of copper-treated plants, it is interesting to note that regrowth only occurred in pots that had been treated exclusively with lead. The addition of copper, at a concentration at or above 500 ppm, seemed to entirely inhibit regrowth. This may be because copper is more toxic to plants than is lead, perhaps especially to the roots. The lead-treated plants may have had some living tissue in the roots, thereby allowing regrowth of the shoots. If copper damaged the roots of the treated plants more severely, the roots may not have been able to support the regrowth of above ground parts.

Our results suggest that lead destroyed all initial above ground parts of the plants but apparently not the roots, which were later able to support the regrowth of shoots in some cases. It could therefore be possible that *L. salicaria* is able to translocate some of the toxic lead ions out of the roots and concentrate them in the shoots, which then die and are later replaced by regrowth from healthy portions of the root. If this process of death and regrowth is allowed to continue, much of the excess lead that is available for up-take by plants will be removed from the soil by the roots of *L. salicaria* plants and transported to the expendable shoots, thus making the soil suitable for the growth of healthy plants. The process of using plants to remove harmful substances from the soil in this way is known as phytoremediation and is a new and promising method of remediation of soils contaminated with metals (Nedelkoska and Doran 2000). There are many advantages to this technique when compared to more traditional forms of remediation, such as minimal destruction of the ecosystem surrounding the treatment area and maintenance of the physical attractiveness of the area and surrounding areas (Nedelkoska and Doran 2000). It has been found that plants that are successful phytoremediators are also hyperaccumulator (Watanabe 1997). These plants are able to absorb large amounts of metals from the environment and are able to concentrate them in their roots, shoots, or leaves (Watanabe 1997). For example, Reeves and others (1983) identified several western North-American genera that accumulate nickel and zinc. If *L. salicaria* is proved to be a hyperaccumulator, it may translocate and concentrate the absorbed metals in its shoots and leaves, given the survival of some of the roots in lead-treated plants observed in this experiment. Further research, using lower concentrations of metals, is required to investigate the feasibility of the use of *L. salicaria* in phytoremediation of soils contaminated with lead and other metals.

ACKNOWLEDGEMENTS. We thank the Research Challenge Grant, EFFRD Program, NASA Opportunities for Visionary Academics, George Gund Foundation, and the Center for Excellence in the Risk Analysis for providing financial support for this research.

LITERATURE CITED

Antosiewicz DM. 1992. Adaptation of plants to an environment polluted with heavy metals. Acta Societatis Botanicorum Poloniae 61:2811-919.

Azar A, Trochimowicz HJ, Maxfield ME. 1973. Review of lead studies in animals carried out at Haskell Laboratory - Two year feeding study and response to hemorrhage study. In: Barth D, Berlin A, Engel R, Recht P, Smeets J, editors. Environmental Health Aspects of Lead. Proceedings of International Symposium, Amsterdam, The Netherlands, October 1972. Commission of the European Communities, Luxembourg. p 199-208.

Baker AJM. 1987. Metal Tolerance. New Phytologist 106:93-111.

Cook CM, Kostedou A, Vardaka E, Lamaras T. 1997. Effects of copper on growth, photosynthesis and nutrient concentration of *Phaseolus* plants. Photosynthetica 34:179-93.

Cooper EM, Sims JT, Cunningham SD, Huang JW, Berti WR. 1999. Chelate assisted phytoextraction of lead from contaminated soils. J Environ Quality 28:1709-19.

Keltjens WG, van Beusichem ML. 1998. Phytochelatins as biomarkers for heavy metal stress in maize (*Zea Mays* L.) and wheat (*Triticum aestivum* L.): combined effects of copper and cadmium. Plant and Soil 293:119-26.

Kjaer C, Elmegaard N. 1996. Effects of copper sulfate on black bindweed (*Polygonum convolulus* L.). Ecotoxicology and Environ Safety 33:110-7.

Kovacevic G, Kastori R, Merkulov LJ. 1999. Dry matter and leaf structure in young wheat plants as affected by cadmium, lead and nickel. Biologia Plantarum 42:119-23.

Kumar P, Dushenkov V, Motto H, Rashkin I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci and Technology 29:1232-8.

Lane SD, Martin ES. 1977. A histochemical investigation of lead uptake in *Raphanus sativus*. New Phytologist 79:281-6.

Luo Y, Rimmer DL. 1995. Zinc-copper interaction affecting plant growth on metal contaminated soil. Environ Pollution 88:79-83.

Mal TK, Adorjan P, Corbett AL. 2002a. Effect of copper on the growth of an aquatic macrophyte, *Elodea canadensis*. Environ Pollution 120:307-11.

Mal TK, Uveges JL, Turk KW. 2002b. Fluctuating asymmetry as an ecological indicator of heavy metal stress in *Lythrum salicaria*. Ecological Indicators 1:189-95.

Mal TK, Lovett-Doust J, Lovett-Doust L. 1997. Time-dependent competitive displacement of *Typha angustifolia* by *Lythrum salicaria*. Oikos 79:26-33.

Mal TK, Lovett-Doust J, Lovett-Doust L, Mulligan GA. 1992. Biology of Canadian Weeds. 100. *Lythrum salicaria*. Canadian J Plant Sci 72:1305-30.

Nedelkoska TV, Doran PM. 2000. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Mineral Engineering 13:549-61.

Ouzounidou G, Eleftheriou E, Karataglis S. 1992. Ecophysical and ultrastructural effects of copper in *Thlaspi ochroleucum* (Cruciferae). Canadian J Botany 70:944-57.

Pahlsson AMB. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air and Soil Pollution 47:287-319.

Punz WF, Sieghardt H. 1993. The response of roots of herbaceous plant species to heavy metals. Environ and Experimental Botany 33:85-98.

Reeves RD, Macfarlane RM, Brooks RR. 1983. Accumulation of nickel and zinc by western North-American genera containing serpentine-tolerant species. Amer J Botany 70:1297-1303.

Romeu-Moreno A, Mas A. 1999. Effects of copper exposure in tissue cultured *Vitis vinifera*. J Agricultural and Food Chemistry 47:2519-22.

Salim R, Al-Subu MM, Atallah A. 1993. Effects of root and foliar treatments with lead, cadmium and copper on the uptake, distribution and growth of radish plants. Environment International 19:393-404.

Sayed SA. 1999. Effects of lead and kinetin on the growth and some physiological components of safflower. Plant Growth Regulation 29:167-74.

Singh RP, Tripathi RD, Sinha SK, Maheshwari R, Srivastava HS. 1997. Response of higher plants to lead contaminated environments. Chemosphere 34:2467-93.

Szalontai B, Laslo IH, Delreczeny M, Droppa M, Horvath G. 1999. Molecular rearrangements of thylakoids after heavy metal poisoning, as seen by Fourier transform infrared (FTIR) and electron spin resonance (ESR) spectroscopy. Photosynthesis Research 61:241-52.

Uveges JL, Corbett AL, Mal TK. 2002. Effects of lead contamination on the growth of *Lythrum salicaria* (purple loosestrife). Environ Pollution 120:319-23.

Vesk PA, Allaway WG. 1997. Spatial variation of copper and lead concentrations of water hyacinth plants in a wetland receiving urban run-off. Aquatic Botany 59:33-44.

Watanabe ME. 1997. Phytoremediation on the brink of commercialization. Environ Sci and Technology 31:182A-6A.

Wierzbicka M. 1999. The effect of lead on the cell cycle in the root meristem of *Allium cepa* L. Protoplasma 207:186-94.

Wilkinson L. 1998. SYSTAT 8.0. SPSS Inc., Chicago, IL, USA.

Wong PK, Chang L. 1991. Effects of copper, chromium and nickel on growth, photosynthesis and chlorophyll a synthesis *of Chloella pyrenoidosa*. Environ Pollution 72:127-39.

Xiong ZT. 1998. Lead uptake and effects on seed germination and plant growth of a Pb accumulator, *Brassica perkinensis* Rupr. Bull of Environ Contamination and Toxicology 60:285-91.

Zhu YL, Zayed AM, Quian JH, de Souza M, Terry N. 1999. Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. J Environ Quality 28:339-44.